ISSN 1585-0404

# Eco-friendly methods to control infection of *Botrytis cinerea* during propagation of grapevines

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***Summary:*** Grey mould (*Botrytis cinerea* Pers.) is a pathogenic fungus which causes damage to the production of grapevine nursery plant materials especially on the stored canes or on graftings during the callusing period. The conditions, increased temperature and humidity, are ideal for the pathogen during the pre-forcing stage and in the storage *Botrytis cinerea* can easily infect the outbursting buds as large amounts of grapevine canes are stored in a relatively small place. The fungicide-based management is general in the prevention of gray rot infection but the palette of authorised chemical compounds is narrowed year by year due to the regulations of the European Union. Our aim is to develop an eco-friendly method which combines the use of natural materials with techniques used in organic farming. Effect of the ultraviolet-c light on the *Botrytis cinerea* was studied. The development characteristics of the pathogen were examined under daylight and dark conditions and experiments were set up with cow's milk and acetic acid. UV-C light destroyed the developed conidia, however, the radiation stimulates the development of immature propagules. Larger quantities of conidiophores and conidia were formed in daylight compared to culture in the dark, while different conditions did not significantly change the mycelial growth characteristics. The developed bacteria prevented the spread of pathogen mycelia during the test with cow's milk in Petri dishes, although the smooth development of propagules that occurred did not change the vitality of the fungal colony. Furthermore the growth of *Botrytis cinerea* fungus mycelia was strongly inhibited by acetic acid.

***Key words:*** acetic acid, *Botrytis cinerea,* cow's milk, different lighting conditions, grapevine propagation, UV-C light

## Introduction

Today 7.5 million hectares of vines are cultivated on Earth. According to the 2012 report of O.I.V. (International Organisation of Vine and Wine) this surface has been reduced to about 260 thousand hectares over the last decade. A similar trend can be observed in Europe and in Hungary (Central Bureau for Statistics, 2012). If we assume that approximately 5–10% of the plantations will be renewed each year, 187,500,000–375,000,000 propagating materials will be needed in case of 2500–5000 vines per hectare, which necessitates the storage of about 50% more grapevine canes.

The fungal pathogen *Botrytis cinerea*, is capable of attacking a large number (proven 235) of host plants and of causing the disease called gray rot (Coley-Smith et al, 1980). Besides the damage caused on vegetables and berry fruits the damage to vines is the most significant. Grey mold infection may cause significant crop losses, and may impair the quality of wine (Martínez et al, 2003).

In the process of the production of propagating materials 1% of damage may already result in a significant loss, not to mention ill-rooted or stunted planting material.

The canes are harvested for the nursery production three-four weeks after natural leaf fall. The canes are then placed in storage rooms until the rooting or grafting process. The *Botrytis cinerea* infection can become widespread in the storage room. There are favourable conditions (high humidity and temperature) after grafting, during the pre-forcing stage for the emergence and spread of the disease, which cause damage to the breaking buds. The plants are concentrated in the storage room, a large number of plants is stored in a small airspace. Fungicides are generally applied in the damage prevention of *Botrytis cinerea*. However, a fungicide resistance has been detected since the 1960s, which was presented in Germany (Kretschme-Hahn, 2008) and in Croatia (Topolovec-Pintari, 2009), therefore the description of the formation process of this resistance has also been attempted (Nagehan-Desen-Nuray-Nafiz, 2012). Because of the fast reproduction of the pathogen and its increasing resistance against fungicides, and also because of the regulations of the European Union that narrow the range of approved chemical agents, the development of an environment friendly procedure has become necessary.

## Material and method

Our tests were divided into several sections. Sampling of *Botrytis cinerea* from *Pelargonium sp*. and *Vitis sp*. (University of Pannonia Faculty of Georgikon Department of Horticulture, Keszthely–Hungary; Georgikon Farm Non-profit Ltd., Cserszegtomaj–Hungary). Cultures were inoculated in Petri dishes (plastic, diameter 93 mm). Potato dextrose agar was used as medium (PDA, Fluka 70139 Potato-dextrose). Grapevine

canes were collected from the experimental vineyard of the Georgikon Farm Non-profit Ltd. (Cserszegtomaj-Hungary). Number of repetitions were 3 (UV-C treatment with *Botrytis cinerea* cultures, treatment with cow milk, acetic acid and different lighting conditions) and 10 (UV-C treatment with infected grapevine canes). 20% strength vinegar was used for the experiment of acetic acid. The treatment was carried out in 1, 2, 3 and 4% dilution. As the control used distilled water and untreated control. Freshly milked cow's milk was collected for the treatment which was cooled to 1–4 ºC (Collected in Georgikon Farm Non-profit Ltd. cattle farm, Keszthely–Hungary) The treatment were carried out in 25, 50 and 100% dilution. Distilled water was used as control.

*UV-C treatment on* Botrytis cinerea *cultures*. The sterilized (Equipment: Steam Sterilizer R23+-type autoclave) PDA was inoculated with *Botrytis cinerea* conidia. The treatment was started after the 7–10 days incubation period when sporulation occurred. The irradiation was carried out with a bulb of 253,7 nm wavelength UV-C light, the lamp had a nominal power output of 30 W based during studies of Nagy (2003), Varga-Haszonits (1987) and Nigro et al. (1998, 2000). The dose was increased by varying the length of the irradiation logarithmically for 1, 3, 10, 30 and 60 minute periods. The distance between the UV-C bulb and cultures or canes was 20 cm. The analysis performed before the irradiation, immediately after irradiation and 7 days after irradiation. For analysis binocular microscope were used (Nikon SMZ800).

*UV-C treatment I. on grapevine canes*. The treatment was carried out with infected canes which were stored in freezer storage room (on 2 ºC degree). The canes were cut with two buds and the basal buds were blinded. The irradiation was performed according to the described parameters. The bulb was placed into a special aluminum cylinder (modified Petri dish disinfectant cylinder). All the 10 canes were evenly irradiated thanks to the aluminum interior which reflects rays of light. The canes were placed into rockwool cubes (Grodan) then irrigated. Finally they were placed under isolation tents.

*UV-C treatment II. on grapevine canes*. The collected canes were stored in freezer storage (temperature 2 ºC) till the treatment. The material was irradiated for 25, 35, 45 and 25+25 minutes long periods. After the irradiation the canes were packed into plastic bags one by one than returned to the storage room. The bags were opened 4 weeks later in a laminar box – also individually and the canes were washed with distilled water. The agars in Petri dishes were inoculated from this suspension and were then closed. These were stored at room temperature. The washed canes were cut two buds pieces and the lower buds were removed. These were placed into rockwool cubes, individually packed in plastic bags and irrigated and then stored in plastic boxes in the forcing room at room temperature.

*Cow*'s *milk treatment*. PDA filled Petri dishes were inoculated with *Botrytis cinerea* conidia. Then immediately were sprayed with cow's milk in concentrations of 25, 50 and 100%. Control cultures were used as comparisons.

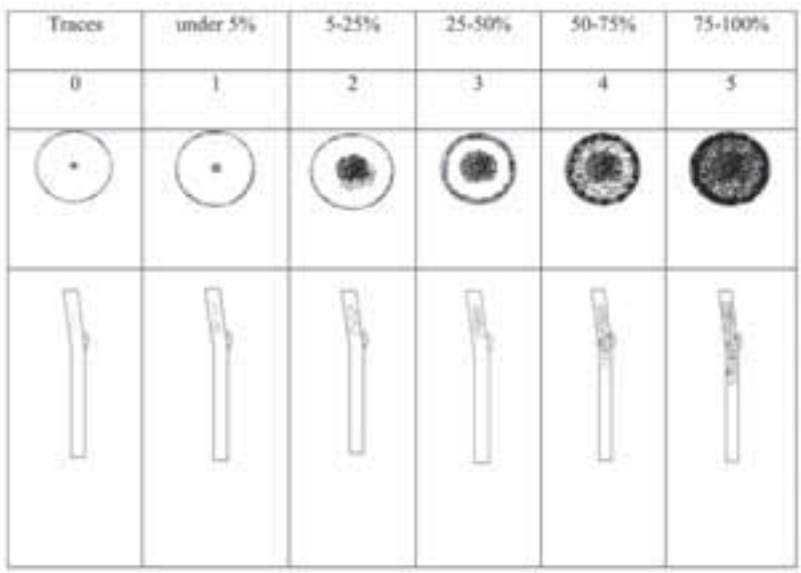
*Acetic acid treatment.* PDA filled Petri dishes were inoculated the pathogen. Immediately after the inoculation the inoculation points were dropped with acetic acid in 1, 2, 3, and 4% concentrations. Control cultures were used as comparisons.

*Observing the effects of different lighting conditions.* The effects of varied light were also studied. PDA filled Petri dishes were inoculated with the conidia of the pathogen. Three group were created. First was wrapped with aluminum foil directly after the inoculation. The second group was wrapped with aluminium foil 24 hours following inoculation. The third group developed in daylight as control.

The results were evaluated by the following methods:

Statement size and vitality of cultures in Petri dishes and on grapevine canes before treatments. Investigate fungi cultures and conidia immediately after the treatment and 7 days later with binocular microscope. The datas was categorized after Townsend-Heuberger disease index (*Table 1.*). Size of cultures from suspension were measured in mm. Cultures after milk, acid and light condition treatments were analized by microscope 7 days later.

***Table 1.*** Categories of Townsend-Heuberger disease index




## Results and discussion

The vitality of cultures did not change from the UV-C light in Petri dishes during the 1, 3 and 10 minutes long treatments. The majority of the conidiophores curled during the 30 minute irradiation. Conidia were damaged. Although the vitality of the cultures considerably decreased, some intact mycelia, conidiophores and conidia remained. After 60 minutes of irradiation some viable conidia were found in the Petri dishes, but most of them were destroyed, the conidiophores were severely damaged (*Figure 1.*). It was found that the treatment stimulated sporulation, Leach (1971)

also made a similar statement on this in relation to *Choanephora cucurbitarum* fungus. Similar results were obtained from infected vine canes.

*Botrytis cinerea* colonies were found only sporadically in the studies which were investigated from suspensions originated cultures since other fungi and bacteria, whose growth was stronger, began to grow in the Petri dishes. However, based on small numbers of comparable cultures it can be established that the pathogen is capable of forming about 30-35 % smaller colonies after 25 to 35 minutes long treatments compared to the control (*Figure 2.*) The grapevine canes started to develop in boxes in the beginning but after a while the leaves were stuck to the bag, and then rotted from the moisture.. The most vigorous canes started rooting but then their surfaces gradually began to get covered by a black slimy coating so further investigation was suspended.

Bettiol (1999) used fresh cow milk against powdery mildew infection of zucchini in green-house when leafs were spreyed with solutions in different dilutions. The study showed that milk was more successful in higher concentration (20-45 %) during control of the infection. Also plantprotectional possibilities were studied against powdery mildew by Medeiros et al. (2012). It was found that cow milk in 30 (v/v) % damaged hyphae and conidiophores. Besides, the microbial community was higher on surface of plants treated with milk than those treated with water or fungicide. During *in vitro* experiments, the vitality of the culture did not change basically during cow milk treatment, but the mycelial growth was hindered by bacterial drops which the pathogen 'evaded' (*Figure 3.*).

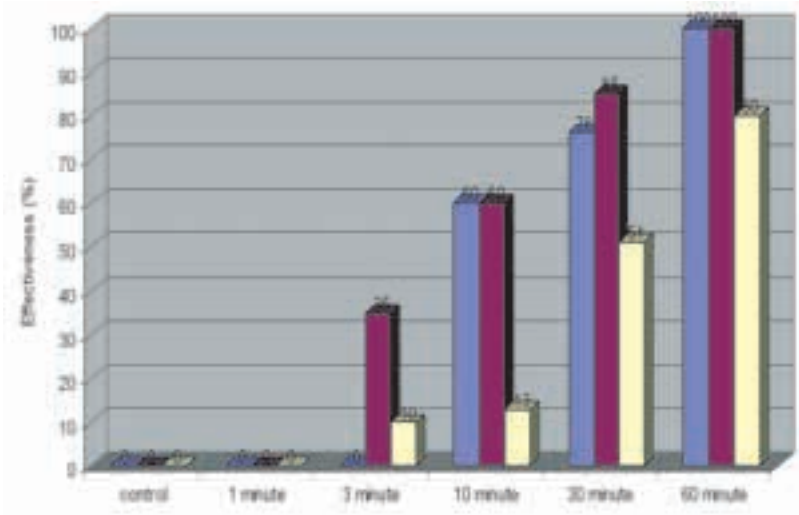



***Figure 1.*** Destroyer effectiveness of UV-C treatment on fungal colonies

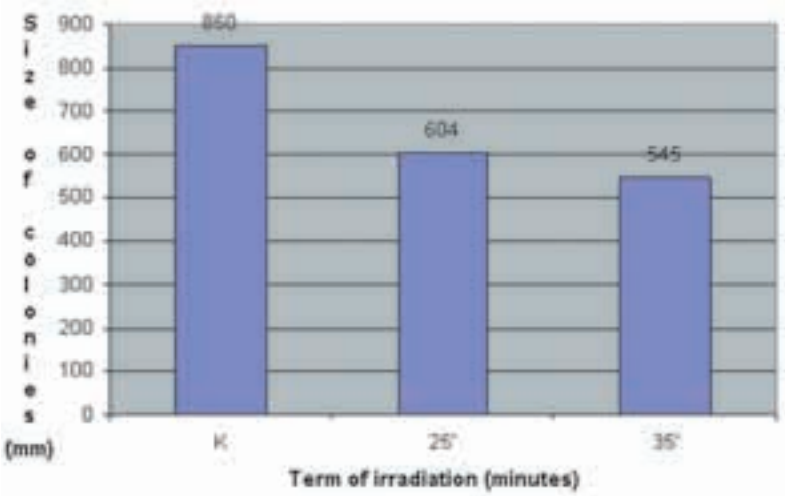



***Figure 2.*** Effect of UV-C irradiation on growth of fungal colonies prepared from suspension

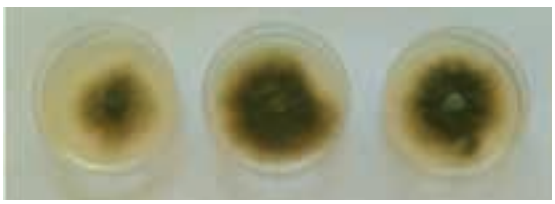
More studies were carried out with acetic acid to decrease postharvest *Botrytis cinerea* infection. Sholberg and Gaunce (1995) studied the effectiveness of postharvest fumigation of apple, tomatoes, grapes, kiwifruit and oranges with acetic acid in concentration of 2,7 and 5,4 mg/L agains fungal infection (*Botrytis cinerea* Pers. and *Penicillium expansum* Link). These pathogens were aimed by Sholberg et al (1996) where the treatment was carried out on table grapes with acetic acid in concentrations of 0,18 (v/v) % and 0,27 (v/v) % on. Both studies reported effectiveness of fumigation to increase the

***Figure 3.*** Impact of cow's milk on the development of *Botrytis cinerea* (Photo by L. FARKAS 2012)


***Figure 4.*** Acetic acid effect on the development of *Botrytis cinerea* (Photo by L. FARKAS 2012))

postharvest rot symptoms on crops caused by *Botrytis* and *Penicillium*. Liu and Chu (2002) studied the effectiveness of acetic acid and thymol against *Monilia fructicola* (G.Wint.) Honey postharvest on apricot and plum. It was found that acetic acid significantly decreased extent of rot. Our studies aimed the control of *Botrytis cinerea* infection on grapevine canes. As a preliminary experiments were carried out the *in vitro* treatment which clearly showed that even a 1% solution inhibited the germination of conidia which did not begin to develop in the least. (*Figure 4.*).

It was visible on cultures between different conditions that the mycelia of the pathogen began to grow in dark or placed into dark 24 hours later. Development of conidia was more viable in daylight than the whole time kept in darkness. While in daylight grown pathogens developed conidia on 47 mm diameter area on average, in darkness they developed 23 mm on surfaces. (*Figure 5.)*



Colonies developed in light.
Colonies developed in darkness.
Colonies developed in darkness 24 hour after inoculation.

***Figure 5.*** Effect of different lighting conditions on development of colonies *Botrytis cinerea*. (Photo by L. FARKAS 2012)

The hour long UV-C treatment kills pathogen propagules and may cause significant damage to the mycelium but further studies are necessary to explore whether wavelength causes permanent damage to the branches. This result is consistent with result of Nigro et al (1998) who demonstrated that the size of *Botrytis* infection and the number of infected berries had decreased. Marquenie et al. (2003) combinated pulsed white light and UV-C light or heat treatment in experiments against *Botrytis cinerea* and *Monilia fructigena*. It was found that the conidia activation decreased when combining UV-C and pulsed light. The solution in our research might be a more powerful bulb with a shorter irradiation time.

New issues arose after the cow's milk treatment which are to be explained including bacterial and fungal allelopathic interactions. The inhibitory effect of the compound on fungal growth was evident during the acid treatment so later it may play an important role in prevention.

The effect of different lighting conditions showed that the pathogen is capable of developing more viable conidia in light and it does not produce so many conidia in the dark. This result may be used when designing storage conditions in such a way that sporulation can be eliminated with different wavelength ranges.

Our results have shown that the combination of treatments may be used for purposes outlined above, and they provide a proper basis for further studies.

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